

## Absence of Functional Mutations in the Ferroportin-encoding *SLC40A1* Gene in Porphyria Cutanea Tarda: A Series of 37 Cases from Southern France

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The presence of iron overload in porphyria cutanea tarda (PCT) is well-known, although the overload is not always concentrated in the liver as was originally thought. The origin of this overload is not known with certainty in all cases and is probably explained by various, most often genetic, mechanisms. From this perspective, the implication of protein activity-modifying mutations or polymorphisms in key genes involved in iron metabolism regulation has already been well documented, with deleterious *HFE* mutations being the most frequently identified changes, occurring in up to one-third of patients (1). Conversely, other genes involved in the regulation of iron metabolism (haemojuvelin, hepcidin, type 2 transferrin receptor) have also been analysed in previous reports with negative results. Ferroportin is another important player in iron metabolism. We report here the search for functionally relevant changes in the ferroportin-encoding *SLC40A1* gene in a series of patients with PCT, the first analysis of its kind.

Thirty-seven consecutive patients diagnosed with PCT based on the usual clinical and biochemical criteria (25 men and 12 women, age range 34–71 years, including 5 patients with familial-type disease), all originating from southern France, were studied. After receiving written consent, genomic DNA was extracted from peripheral blood mononuclear cells and subjected to *SLC40A1* analysis using stringent amplification conditions. Then, its eight exons and flanking areas were directly sequenced. The *HFE* gene was also analysed for functionally significant mutations (homozygous or heterozygous C282Y mutations and the homozygous H63D mutation). (The heterozygous H63D mutation was not studied since its possible effect on iron metabolism remain doubtful and it is frequently encountered in the Mediterranean basin.)

Analysis of the *HFE* and *SLC40A1* genes was performed in all 37 patients. In the case of *SCL40A1*, a number of known polymorphisms with no influence on the primary structure of the encoded protein were identified. A previously unreported complex genomic change was identified in one male patient with familial PCT. This change caused intron 6 to start at nucleotide 760 and involved the insertion of 4 nucleotides, deletion

of 16 nucleotides and duplication of 18 nucleotides. *HFE* gene analysis revealed a homozygous or heterozygous C282Y mutation or homozygous H63D mutation in 9/37 patients (24%). It is notable that the patient with the complex *SCL40A1* changes did not carry any relevant *HFE* mutations, genetic analysis revealing only a heterozygous H63D mutation, that is usually not considered to cause functional consequences or significant iron overload (normal ferritin level and magnetic resonance imaging-measured liver iron content).

Ferroportin plays an important role in the unidirectional export of iron from cells to extracellular structures. In enterocytes, ferroportin and haephaestin together export iron through the basolateral membrane and then into the plasma. Ferroportin also plays a role in iron flow from the cell interior to the extracellular medium in macrophages, without the assistance of haephaestin. To date, two types of *SLC40A1* mutations with effects on protein function that lead to iron overload have been described (2). One, involving loss of its ability to export iron, causes defective localization of the protein on the cell surface and subsequent iron sequestration, mainly in macrophages, resulting in typical ferroportin disease with low transferrin saturation and early Kùpffer cell iron overload. In the other, the mutation does not modify ferroportin's intrinsic activity, but confers on it resistance to inhibition by hepcidin and thus increases intestinal absorption of iron, causing high transferrin saturation and early hepatocyte iron overload similar to that which occurs in classic hereditary haemochromatosis. Overall, our study does not favour the hypothesis of a significant involvement of ferroportin changes in iron overload in patients with PCT, since only one complex change, potentially deleterious for protein function but seemingly not the cause of iron overload, was identified in a series of 37 cases.

## REFERENCES

1. Alla V, Bonkovsky HL. Iron in nonhemochromatotic liver disorders. *Semin Liver Dis* 2005; 25: 461–472.
2. De Domenico I, Ward DM, Musci G, Kaplan J. Iron overload due to mutations in ferroportin. *Haematologica* 2006; 91: 92–95.